Title	QUALITATIVE AND QUANTITATIVE ASSESSMENT OF REMINERALIZING EFFECT OF PROPHYLACTIC TOOTHPASTE PROMOTING BRUSHITE FORMATION: A RANDOMIZED CLINICAL TRIAL
Methodology	Randomized, controlled trial, blind study
Study Duration	Estimated duration for the main protocol (e.g. from start of screening to last subject processed and finishing the study) is approximately 6 months.
Study Center(s)	Institute of Dentistry, Sechenov University, Moscow, Russia
Objectives	Primary: to assess the decrease in teeth hypersensitivity after the use of brushite-containing toothpaste; Secondary: to evaluate the increase in enamel acid resistance and speed of its remineralization after the use of brushite-containing toothpaste.
Number of Subjects	54 randomized patients in three arms; Study, Positive Control and Negative Control
Diagnosis and Main Inclusion Criteria	Inclusion criteria: Male and female patients, 20-25 years old, in any distribution, with enamel hypersensitivity. Exclusion criteria: the presence of orthodontic appliance, malocclusion, allergic reactions to the components of toothpastes, systemic diseases, pregnancy.
Study Product, Dose, Route, Regimen	Group 1 used toothpaste promoting the formation of brushite (test group). Group 2 used toothpaste with hydroxyapatite (positive control group). Group 3 used toothpaste without fluoride and without hydroxyapatite (negative control group).
Statistical Methodology	Simplified Oral Hygiene index (OHI-S), enamel acid resistance test, rate of enamel remineralization, Schiff test for the assessment of enamel sensitivity.

Purpose:

The primary objective is to assess the effect of toothpaste, forming a brushite, on the functional acid resistance of enamel and the speed of its remineralization.

Background:

Tooth hypersensitivity is a prevalent condition among the population. It is one of the clinical manifestations of enamel demineralization. Different remineralizing agents are used for the prevention of further demineralization and the decrease of hypersensitivity. The main components of these products are Ca, P, and F ions which aid the restoration of enamel structure, as the deficiency of these ions weakens the enamel. Currently various studies are conducted to improve the effect of existing and developing new remineralizing agents. One of the trends in remineralizing therapy is the development of toothpaste allowing brushite crystals formation in the demineralized lesions of hard tooth tissues.

Goals of the study:

1. To evaluate oral hygiene status in patients with teeth hypersensitivity as measured by the Simplified Oral Hygiene index (OHI-S) in the study and control groups at baseline, at the different timepoints of the study.
2. To evaluate the level of enamel mineralization in patients with teeth hypersensitivity as measured by the enamel acid resistance test in the study and control groups at baseline, at the different timepoints of the study.
3. To evaluate the rate of enamel remineralization in patients with teeth hypersensitivity in the study and control groups at baseline, at the different timepoints of the study.
4. To evaluate the tooth hypersensitivity in patients in the study and control groups as measured by the Schiff test at baseline, at the different timepoints of the study.

Duration of the Study:

The study is estimated to complete enrollment within 1 month from study initiation; however, enrollment will remain open until the study goal is met. The duration of this study for each subject will be a maximum of four (4) weeks.

Product Description:

Three toothpaste will be used in the study.

The composition of the toothpaste used by the test group:

Bottle number 1 composition: calcium nitrate, sorbitol, glycerin, drinking water, silica, aromatic composition, xanthan gum, xylitol, nettle extract, chamomile extract, yarrow extract, calendula extract, sage extract, echinacea extract, stevioside, D-panthenol, sodium benzoate, potassium sorbate, papain, and natural indigo dye.

Bottle number 2 composition: ammonium hydrogen phosphate, sorbitol, glycerin, drinking water, silica, aromatic composition, xanthan gum, xylitol, green tea extract, aloe vera extract, calamus extract, menthol, stevioside, D-panthenol, allantoin, sodium benzoate, potassium sorbate, natural dye, and beta carotene.

The composition of the toothpaste used by the positive control group:

Aqua, Sorbitol, Hydrated Silica, Glycerin, Hydroxyapatite 6% (nano), Cellulose Gum, Sodium Myristoyl Sarcosinate, Sodium Methyl Cocoyl Taurate, Aroma, Xanthan Gum, Stevia Rebaudiana Extract, Anethole, Tetrasodium Glutamate Diacetate, Tocopheryl Acetate, Eucalyptol, o-Cymen-5-ol, Citric Acid, Vitis Vinifera (Grape) Seed Extract, Tannase, Thymol, Limonene. Fluoride free.

The composition of the toothpaste used by the negative control group:

Hydrogenated Starch Hydrolysate, Hydrated Silica, Aqua, Glycerin, Cellulose Gum, Dipotassium Glycyrrhizate, Polyglyceryl-4 Laurate/ Sebacate, Polyglyceryl-6 Caprylate/Caprate, Xanthan Gum, Menthyl Lactate, Perilla Frutescens Seed Extract, Colloidal Silver.

Product Intended Use:

All the three toothpastes are intended for daily oral hygiene procedure and are over the counter.

Methods:

Study Design.

Sixty people aged 20–25 years old were examined and randomly assigned to one of the study groups:

Group 1 used toothpaste promoting the formation of brushite (test group).

Group 2 used toothpaste with hydroxyapatite (positive control group).

Group 3 used toothpaste without fluoride and without hydroxyapatite (negative control group).

The allocation concealment was performed by the use of containers numbered by a “third party” (person, who didn’t participate in the study). The toothpastes in white bottles without any titles were placed in the containers. The weight of the pastes and bottles in different groups was the same. The patient on enrolment received a sealed container with a toothpaste. Neither patients nor researchers were aware of the type of a toothpaste received by each patient.

Observational Parameters:

The patients used the prescribed pastes for a month. Control examinations were carried out in the following periods: 2 weeks and 4 weeks.

The examination included:

Simplified Oral Hygiene index (OHI-S),

enamel acid resistance test [16],

rate of enamel remineralization [17],

Schiff test for the assessment of enamel sensitivity.

For the assessment of enamel acid resistance, a drop of hydrochloric acid buffer solution (pH: 0.3-0.6) was applied on the buccal surface of maxillary central incisor (middle third) for a minute. After that the acid was rinsed off with water, and 2% methylene blue aqueous solution was applied for five minutes. The assessment of staining intensity was performed using ten-point printed scale.

To evaluate the rate of enamel remineralization, the staining was repeated daily until the loss of staining ability. The number of days was fixed and served as a quantitative parameter of enamel remineralization rate.
Schiff test was performed to assess enamel sensitivity in all groups as follows: air from air/water syringe was applied perpendicular to the cervical areas of all teeth from a distance of 1 cm for a second. Enamel sensitivity was estimated in accordance with the following criteria:
0 - no reaction,
1 - discomfort but the patient does not insist on stopping the test,
2 - discomfort, accompanied by a request to discontinue the test,
3 - severe pain reaction with pronounced motor reactions aimed at the immediate termination of the test.

Data collection and reporting.
Data will be collected at the following points: at baseline, and after 2 and 4 weeks of the assigned toothpastes use.
Data from the study will be maintained for two (2) years after the date the investigation is completed, terminated or until the records are no longer required to support the protocol, whichever date is later. Patient records and data are eligible for inspection and/or copying by applicable regulatory authorities.

Expected outcomes.
It is the expectation that both hydroxyapatite and brushite-containing toothpastes will show improved patient outcomes over negative control. Improvements in tooth hypersensitivity, enamel acid resistance, and remineralisation rate are expected from the study and positive control groups compared to the negative control group. Data interpretation and the statistical significance of the results will be described later in this protocol.

Adverse reactions.

There is no expectation of any adverse outcomes or reactions due to a patient using the assigned toothpastes. However, hypersensitivity reactions to some of the toothpastes' components are still possible. All participants will be given access to contact info of the investigators. Any adverse reactions should be reported immediately to the investigators.

Reasons for Withdrawal or Termination

A subject may be discontinued from the study at any time if the subject or the Investigator feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

• Screening Failure
• Subject withdrawal of consent
• Subject is not compliant with study procedures
• Adverse Event that in the opinion of the Investigator would be in the best interest of the subject to discontinue study participation
• Protocol violation requiring discontinuation
• Lost to follow-up

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice. Reasonable attempts will be made by the Investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents and the Case Report Form (CRF). If a subject is withdrawn from treatment due to an AE (adverse Event), the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized. The Investigator must make every effort to contact subjects who are lost to follow-up. Attempts to contact such subjects must be documented in the subject's records (e.g., times and dates of attempted telephone contact, receipt for sending a registered letter, etc.).

Handling of Participant Withdrawals of Termination:

Although subjects may withdraw from the study at any time and for any reason, (or may be withdrawn at the Investigator's discretion), subject withdrawal should be avoided as much as reasonably possible. In any case, appropriate follow-up for endpoints should be continued. Subjects who prematurely discontinue are not to be replaced. For subjects considered lost to follow-up, the CRF must be completed up to the last visit performed.

Premature Termination or Suspension of Study:

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the Investigator and the Institutional Review Board (IRB), as appropriate. If the study is prematurely terminated or suspended, the Investigator will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination or suspension include, but are not limited to:

• Determination of unexpected, significant, or unacceptable risk to participants
• Demonstration of efficacy that would warrant stopping
• Insufficient compliance to protocol requirements
• Data that are not sufficiently complete and/or evaluable
• Determination of futility

Study may resume once concerns about safety, protocol compliance, data quality are addressed and satisfy the IRB.

Methods and Study Schedule:

Subjects eligible for the study will review and undergo informed consent. Once consented, subjects will be randomly assigned on a 1:1:1 basis to undergo:

- Group 1 to use toothpaste promoting the formation of brushite (test group).
- Group 2 to use toothpaste with hydroxyapatite (positive control group).

- Group 3 to use toothpaste without fluoride and without hydroxyapatite (negative control group).

Baseline/Screening Visit (-61 to -1 days from Day 0)

The following procedures will be performed at the Baseline/Screening visit:

- Review the study with the subject and obtain written informed consent
- Assign the subject a unique screening/enrollment number
- Review and record medical history, and medication history to determine eligibility based on inclusion/exclusion criteria
- Record demographics (age, race, ethnicity, gender)
- Document vitals
- Document all current medications, including medications over-the-counter and herbal medications
- Perform physical examination
- Administer OHI-S, enamel acid resistance test, enamel remineralization rate, and Shiff test.
- The allocation concealment will be performed by the use of containers numbered by a “third party” (person, who will not participate in the study). The toothpastes in white bottles without any titles will be placed in the containers. The weight of the pastes and bottles in different groups will be the same. The patient on enrolment will receive a sealed container with a toothpaste. Neither patients nor researchers will be aware of the type of a toothpaste received by each patient (double blinding).

The following standard of care techniques will be used according to the arm the patient is randomized to:

Negative Control Group (blind):

The eighteen (18) member negative control group will receive oral hygiene instructions (including the use of the assigned toothpastes twice daily, brushing technique, the use of interdental flosses and/or brushes). The examination will include:

- Simplified Oral Hygiene index (OHI-S),
- enamel acid resistance test,
- rate of enamel remineralization,
- Schiff test for the assessment of enamel sensitivity.

Positive Control Group (blind):

The eighteen (18) member positive control group will receive oral hygiene instructions (including the use of the assigned toothpastes twice daily, brushing technique, the use of interdental flosses and/or brushes). The examination will include:

- Simplified Oral Hygiene index (OHI-S),
- enamel acid resistance test,
- rate of enamel remineralization,
- Schiff test for the assessment of enamel sensitivity.

Study Group (blind):

The eighteen (18) member study group will receive oral hygiene instructions (including the use of the assigned toothpastes twice daily, brushing technique, the use of interdental flosses and/or brushes). The examination will include:

- Simplified Oral Hygiene index (OHI-S),
- enamel acid resistance test,
- rate of enamel remineralization,
- Schiff test for the assessment of enamel sensitivity.

For the assessment of enamel acid resistance, a drop of hydrochloric acid buffer solution (pH: 0.3-0.6) was applied on the buccal surface of maxillary central incisor (middle third) for a minute. After that the acid was rinsed off with water, and 2% methylene blue aqueous solution was applied for five minutes. The assessment of staining intensity was performed using ten-point printed scale.

To evaluate the rate of enamel remineralization, the staining was repeated daily until the loss of staining ability. The number of days was fixed and served as a quantitative parameter of enamel remineralization rate.

Schiff test was performed to assess enamel sensitivity in all groups as follows: air from air/water syringe was applied perpendicular to the cervical areas of all teeth from a distance of 1 cm for a second. Enamel sensitivity was estimated in accordance with the following criteria:

0 - no reaction,

1 - discomfort but the patient does not insist on stopping the test,

2 - discomfort, accompanied by a request to discontinue the test,

3 - severe pain reaction with pronounced motor reactions aimed at the immediate termination of the test.

Follow-up visits (up to 6, daily after the baseline visit) - all groups

- rate of enamel remineralization assessment
- assess for adverse events

Second visit (2 weeks from the baseline visit) - all groups

- Simplified Oral Hygiene index (OHI-S),
- enamel acid resistance test,
- rate of enamel remineralization,
- Schiff test for the assessment of enamel sensitivity.

- Assess for adverse events

Follow-up visits (up to 6, daily after the second visit) - all groups

- rate of enamel remineralization assessment
- assess for adverse events

Third visit (2 weeks from the second visit) = Final Study Visit - all groups

- Simplified Oral Hygiene index (OHI-S),
- enamel acid resistance test,
- rate of enamel remineralization,
- Schiff test for the assessment of enamel sensitivity,

- Assess for adverse events

Early Termination

All subjects have the right to withdraw from study participation at any time during the study. If, for whatever reason, a subject withdraws from the study, an Early Termination visit will be performed.

The following procedures will be performed (if agreed upon by the subject) at the Early Termination visit:

- Assess for adverse events
- Assess for complications following treatments
- Document all current medications, including medications over-the-counter and herbal medications

RANDOMIZATION

Subjects who meet all inclusion and exclusion criteria will be randomized on Treatment Day in a 1:1:1 ratio to either the Study Group or the Positive or Negative Control Group, in accordance with a computer-generated schedule prepared by a third-party person, who will not participate in the study. Study personnel will be instructed not to randomize until subject has been confirmed to meet all inclusion/ exclusion criteria on treatment day.

SAMPLE SIZE JUSTIFICATION

Sample Size Calculations

The sample size was determined according to the previous trial by the same authors, where Shiff index was assessed. The power was set at 80%, alfa-level was set as 0.05, the means from the previous study were 2.6±0.75 and 1.9 in toothpaste and placebo groups, respectively. The allocation ratio was equal to 1. The target sample size comprised 18 participants in each group, 54 patients total.

STATISTICAL ANALYSIS PLAN

Primary Endpoint

Tooth hypersensitivity as measured with Shiff's index

Secondary Endpoints

Enamel mineralisation characteristics as measured by enamel acid resistance test and rate of enamel remineralisation.

All analyses will be performed using per-protocol population as well as intention-to-treat population (to include subjects who are withdrawn prematurely or randomized but not treated per randomization arm).

ASSESSMENT OF SAFETY

Adverse events (AE) will be monitored and collected by the study team from the point of signed consent until 7 (for non-serious AEs) or 30 days (for serious AEs, SAEs) after the last day of study participation. For each AE, a detailed explanation will be obtained from the subject and subject's medical record. All AEs will be recorded on the CRFs.

Definition of Adverse Event

An AE is defined as any unanticipated medical occurrence regardless to relationship of the investigative arm of the trial. An AE can be any unintended sign, lab abnormality, symptom, or disease associated with the trial. Any abnormality that presents during a medical test are to be defined as an AE if it produces clinical signs and/or symptoms, requires intervention, or deemed clinically significant by the Investigator.

Pregnancy

If a female subject becomes pregnant during the trial, she must be followed up until the outcome of the pregnancy is known. The outcome of the pregnancy must be reported to the Investigator on the appropriate AE CRF.

DATA MONITORING

The Principal Investigator will be responsible to ensure the study is conducted in accordance with the protocol, Good Clinical Practice (GCP), applicable regulatory requirements, and that the data recorded is valid. To achieve this objective, the

study will be continuously monitored and reviewed on a monthly basis by the study team.

Clinical site monitoring is conducted to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, and with applicable regulatory requirement(s).

A Clinical Monitoring Plan will be created by the Sponsor and describe in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

DATA HANDLING AND RECORD KEEPING

The collection of personal patient information will be limited to the amount necessary to achieve the aims of the research, so that no unneeded sensitive information is being collected.

Only study personnel will collect data. Hard copy documents will be retained for the duration of the study until data entry. All hard copy documents will be kept in a locked cabinet in the research coordinator’s office. Data entry will be exported into Excel file format (password protected), which will then be used for data analysis. Only de-identified data will be used for data analysis. All hard copy documents will be shredded within five years after completion of the study.

Collected de-identified data will be sent to a biostatistician for statistical analysis.

INSTITUTIONAL REVIEW BOARD

The protocol, informed consent form(s), and all participant materials will be submitted to the IRB for review and approval. Approval must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

CONSENT PROCESS

Subjects will be approached when they come through the Dental center for any type of treatment. Each potential subject must provide written consent with full knowledge of the procedures involved. The informed consent, approved by the IRB and in accordance with regulatory guidelines, must be fully explained by the Investigator or member of the study staff including the study aims, methods, benefits and risks, and signed by the subject before enrollment into the study. Potential subjects will be informed that study participation is voluntary and that they may withdraw at any time. The subjects will be told that choosing against participation will not affect the care received for treatment. The subjects will be informed that they will be authorizing access of investigational staff to confidential medical records. The subject will be given sufficient time to read the consent and ask any questions. Once the informed consent is signed, the subject will be given a copy of the document.

PROTOCOL DEVIATION

A protocol deviation is any noncompliance with the clinical trial protocol or GCP requirements. The noncompliance may be either on the part of the participant, the Investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

All protocol deviations/violations should be documented using the Protocol Deviations/Violations CRF and submitted to the IRB according to their reporting guidelines.

LAWS AND REGULATIONS

This clinical study will be conducted in compliance with all national laws and regulations of the countries in which the clinical trial is performed, as well as any applicable guidelines. The trial will be registered on http://www.isrctn.com and on other sites, as appropriate.

PUBLICATION AND DATA SHARING POLICY

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws.

STUDY PERSONNEL:

1. Maria A. Polyakova, Phd, assistant professor at the department of therapeutic dentistry in I.M. Sechenov First Moscow State Medical University.
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6. Vladlena Yu. Doroshina, Phd, associate professor at the department of therapeutic dentistry in I.M. Sechenov First Moscow State Medical University.
7. Nina Y. Novozhilova , Phd, associate professor at the the department of therapeutic dentistry in I.M. Sechenov First Moscow State Medical University.

The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Sponsor and documented approval from the Institutional Review Board, except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.